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EDITORIAL

SCIENCE, SENTIMENT AND ECONOMICS

THE work of the veterinary profession is often compared and likened to that of the sister profession which has to do with the human species. Indeed, there are many points of similarity. Those who would enter these professions are of similar type and imbued with the same urge. The preliminary educational standards are the same and the course of instruction is almost identical in its plan. In the practice of the two arts there is a fundamental difference, but not in the purely scientific sense, i.e., the methods employed for the diagnosis and treatment of disease, for in one case sentiment and the sacredness of human life governs action; in the other economic factors play the major role.

By and large a veterinary surgeon can only justify his existence if his work is of economic value to his client.

Medical men are not subject to such repression, for every effort must be made to keep the flame of life alight, even in those cases where there is no hope of cure and even if the patient is in great misery and is an encumbrance and distress to those around him.

It is well to bear these facts in mind. There may be some who think it is wrong that our efforts to alleviate and cure the diseases of domesticated animals should be subject to restrictions of an economic nature. They may argue that a man whose scientific scope is limited by mundane questions of pounds, shillings and pence, and who cannot pursue his therapeutic ideas to an ultimate and conclusive end, is bound to suffer frustrations which will stultify the advance of knowledge.

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In a rigid academic sense this may be true, but there are compensations which more than outweigh many of the disadvantages.

To a practising veterinary surgeon there are many calamities which are worse than the death, natural or otherwise, of his patient.

It is not necessary to keep a useless, suffering or inefficient beast alive to be a burden on its owner or to propagate a race of C_3 animals. A veterinary surgeon can exercise his judgment as to what should be preserved or discarded. It is this factor which has been responsible for the evolution of the magnificent stock which are our pride to-day and which have made these islands the nursery of the best animals in the world. There are occasions also when this power to terminate life can be used to good purpose in the control of disease, especially those diseases of a highly contagious nature and which are prone to develop to an epidemic form.

The slaughter policy has been said to be the refuge of those who are scientifically destitute.

In terms of pure academic science this may be true, but there is no doubt that it gives good results in the field. Whether one takes a long or a short view, there can be no doubt that, under conditions such as are met with in these islands, it pays good dividends economically, and the sacrifice of the few limits much misery and suffering amongst the many.

One of the disadvantages of modern medical science is that many men and animals are preserved only to pass on to future generations physical and genetical defects which may lead to a deterioration of the species.

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GENERAL ARTICLES

JOINT-ILL (PYOSEPTICÆMIA) IN A HACKNEY FOAL DUE TO A HÆMOLYTIC STREPTOCOCCUS AND ITS TREATMENT WITH PENICILLIN AND SULPHAMEZATHINE

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Introduction

SINCE it was first shown that joint-ill (pyosepticæmia), at any rate in part, resulted in a primary infection via the navel (Bollinger, 1873) the cause of the disease has been, step by step, elucidated by various workers in this country, in North America, and on the Continent. In the past the disease has occupied a prominent part in veterinary literature, but since mechanisation has largely replaced the horse, there has been a tendency for foal diseases to become unduly neglected.

While pyosepticæmia is a disease common to the new-born of various species, foals appear particularly susceptible, and it is probably responsible for more deaths than any other disease. According to Udall, the mortality in the acute form occurring a few hours after birth is 90-100 per cent, and the general average death rate has been estimated at 30 to 75 per cent.

The disease may be caused through a number of different organisms and become acutely fatal, sub-acute or chronic according to the infection concerned. Gaiger and Davies give the incidence in Great Britain as follows:—

Streptococci—in 40-50 per cent of cases.

Shigella viscosa (*Shigella equirulis, Bacillus nephriditis equi., B. viscosum equi.*—25 per cent of cases.

Escherichia coli.—25 per cent of cases.

Salmonella abortivo-equinus—5 per cent of cases.

Diplococci, Corynebacterium pyogenes, etc.—5 per cent of cases.

It is characterised by a generalised septicæmia, and in the less acute forms suppuration of the umbilicus, joints and tendon sheaths and a tendency to metastatic abscess formation in other parts of the body. In *Shigella viscosa* infections the kidneys show characteristic changes.

While knowledge of the ætiology came, unfortunately, late in the day, knowledge of satisfactory treatment came even later, and to-day appears only in its infancy. Of the two cases recorded, one occurred before the availability of penicillin and sulphamezathine. The other at a time when stocks were just becoming fairly plentiful.

History, Course and Treatment

The subject was a pure-bred Hackney colt foal, three weeks old. It was first seen on June 7, 1950. It had an abscess discharging at the brisket and

also a swelling of the off stifle and fetlock joint. It was markedly lame and had a temperature of 103° F.

It was given half a million units of procain penicillin in oil and 30 c.c. of sulphamezathine 33⅓ per cent, both subcutaneously.

On the following day there was a considerable improvement, the foal practically going sound and the brisket abscess discharging less. The temperature was, however, unchanged, so the treatment was repeated and the swollen joints rubbed with a painless liquid blister.

On the third day the penicillin was reduced to 300,000 units and the sulphamezathine to 25 c.c. The liquid blister was repeated on the swollen joints.

By the fifth day there was a wonderful improvement. The foal was going sound and lashed out at the mare while walking to the loose box. Improvement was so satisfactory that in view of economy it was decided not to see the foal again, but as a preventative measure 300,000 units of penicillin were again injected.

June 21.—Ten days later a further request was received to see the foal. The stifle and fetlock were again swollen. Both the mare and the foal were exceptionally wild, and as they had a wide range of marshland to roam it was obvious the foal's joints were receiving considerable abuse. Unfortunately, it was not convenient for the owner to house the mare. The penicillin treatment was repeated and a dose of mixed vaccine given.

The following day there was again amazing improvement. The foal was galloping with the usual playful ways of a normal foal. It was therefore decided that it would do him more harm than good to chase him into a box for further treatment.

July 7.—Just over two weeks from the last visit, yet a further request came to see the foal. It was obvious that the animal had been going down-hill some time and should have been attended earlier.

The owner had now, however, lost interest, and was anxious for destruction. The foal had lost its bloom, was losing weight, and was beginning to show that gaunt, tucked-up, tight-skinned appearance all too typical of the disease. It spent most of the time recumbent, and when raised was disinclined to move and swinging the affected leg from the pelvis. This was aptly described by the owner: "It looks as if the leg is broken." The stifle was greatly distended with fluid and extremely painful. From considerable experience with similar cases over a number of years, the condition looked absolutely hopeless. It was, however, the disappointment after the previous remarkable recovery which caused the writer to prevail upon the owner to continue treatment a day or two longer.

Recalling that Auchterlonie had recommended aspiration and injection directly into the joint capsule, this method of treatment was adopted. After clipping and cleansing, a fairly large bore needle was inserted into the most pendulous part of the swelling and as much fluid as possible was drained away, after which half a million units of oily procain penicillin were injected—some through the original puncture and some in other points into the joint capsule. In addition, 30 c.c. of sulphamezathine solution were injected subcutaneously.

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July 9.—Forty-eight hours after, the treatment was repeated. The swelling was noted to be less and the foal was noticed once or twice to use the affected leg when walking.

The following day the foal rose with alacrity and the original puncture wound had healed, showing the normal stifle groove. There was, however, still fluid lower down, towards the hock point. This was aspirated and the injections continued at the lowest point.

This technique was again repeated on two successive days, July 11 and 12. By this time the foal was again using the limb to a remarkable degree, and by July 13 the foal was walking almost sound, and from this time onwards the recovery was uneventful.

A swab from the original abscess taken on June 7 and a sample of fluid aspirated from the stifle joint on July 7 were both submitted to the Institute of Animal Pathology, Cambridge, which reported in both cases the presence of a hæmolytic streptococcus, probably Group C *Str. equi.*

Discussion

Looking back to treatment of a similar case in the old days, a swelling of one joint was indeed an omen for a serious prognosis.

The encroachment of infection to a further joint was invariably considered the beginning of the end. The extremely exceptional case where the foal did possess the vitality and constitution to struggle through was usually an unthrifty wreck for months. To those who have experienced the successive heavy mortality and relentless toll this new method of therapy stands out as remarkable and revolutionary. There is little doubt in this case that but for the attempted economy in treatment, and with the larger doses of penicillin as now used, the final result would have been quicker and the relapses avoided.

Acknowledgments

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AN ATYPICAL CASE OF PYOSEPTICÆMIA IN A FOAL WHICH REVEALED A HÆMOLYTIC (GROUP C) STREPTOCOCCAL INFECTION

The subject was a pedigree Suffolk foal, five weeks old. It was first seen on July 7 (during the war years). The history of the case is as follows: Before my examination it had been slightly lame for several days, and was about four weeks old before symptoms were noticed. When seen it had a temperature of 104° F., a swelling on the ribs behind the near elbow, a swelling of the off elbow joint, and a swollen stifle joint on the same side. The foal was injected intramuscularly with Prontosil (Bayer) and the stifle rubbed with a painless liquid blister. On the following day the stifle was normal, but the swelling had gone

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down to the hock with œdema to the fetlock. The off knee and elbow regions were much more swollen and the temperature had risen to 104.5° F. A further dose of Prontosil was given and the foal put on sulphanilamide and cod-liver oil electuary. The abscess on the ribs was blistered and the other swellings fomented. The treatment was continued until July 18, approximately 10 days. At this time the abscess on the ribs was ripe, and a sample of its contents was collected under aseptic conditions, and a laboratory examination revealed hæmolytic (Group C) streptococcal infection. The remarkable point was that during this period the foal was not acutely lame, kept fairly bright, and kept the mare's udder well drawn out.

The condition improved and an autogenous vaccine was prepared. Unfortunately, the day before the first dose of vaccine there was a flare-up of the swelling, this time the near hind leg, with an œdematous, tied-off swelling from the hock downwards. This appeared to be too much for him and he died on August 7, almost five weeks since the first symptoms were noticed.

Post-mortem examination showed extensive infection throughout. The navel showed inspissated pus extending to the liver. The liver was yellow and degenerated. The lungs were hepatised and millett seed-like nodules could be palpated throughout the lung substance. The kidneys seemed very large and the small bowel showed acute inflammation.

A kidney and portions of the liver and lung were forwarded to the laboratory, which reported that a bacterial examination of the specimen received revealed a great deal of contamination, but that hæmolytic streptococci were demonstrated in the tissues.

Discussion

The first reason for recording this case is the unusual form in which most of the limb joints became affected. In spite of this, although the temperature was abnormally high, there was no acute lameness, no pointing of the joints to end in open joint, and no constitutional symptoms severe enough to prevent the foal from completely emptying the udder of its dam. There was, also, considerable œdema of the limbs, with tied-off swellings which rather suggested purpura instead of joint-ill, yet in the abscess during life and the tissues after death a hæmolytic streptococci was isolated.

This is in contrast to our usual experience where one joint is primarily affected with acute lameness. When infection finally extends to other joints, this usually appears as a sign that the defence forces of the body have been overcome, and, when this happens, within a few days death usually supervenes.

The second reason for recording the case is that it occurred at a time when penicillin was unobtainable for veterinary use and before sulphamezathine became available. It therefore appears to form a comparison with the Hackney foal which had the advantage of this treatment. Both cases possessed several similarities. They both became affected unusually late after birth, both had a streptocci infection, they occurred on adjoining farms, and both were cases which from past experience one would expect to end fatally. It is true the Suffolk foal had the advantage of the earlier sulphonamide treatment, but in spite of

this it took the fatal course we had come to accept as the final outcome.

In view of the other case recorded, it seems extremely probable that if a similar line of therapy could have been adopted here, there would have been a good prospect of a quick and uneventful recovery.

Acknowledgments

The writer wishes to express his thanks to Prof. F. Blakemore, of the Institute of Animal Pathology, Cambridge, for carrying out the laboratory examinations.

SELECTIVE BACTERIOSTATIC SUBSTANCES FOR THE ISOLATION AND CULTIVATION OF *ASTEROCOCCUS BOVIS*, THE CAUSAL ORGANISM OF CONTAGIOUS BOVINE PLEUROPNEUMONIA

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THE action of selective bacteriostatic substances on pleuropneumonia-like organisms from human and animal sources has been described by several authors. Edward (1947, 1950) tried penicillin, thallium acetate, sodium azide, gentian violet, brilliant green, and potassium tellurite, and found the first two of value; Beveridge (1943) recorded the use of sulphanilamide; and Smith, Morton, and Leberman (1950) tested sodium azide, brilliant green, nile blue, potassium tellurite, crystal violet, and basic fuchsin, the last three of which were found by these authors to be useful.

Mornet, Ballis, and Bachiron (1949), working with *Asterococcus bovis*, the causal organism of contagious bovine pleuropneumonia, found penicillin without effect on this organism, while streptomycin and tyrothricin were bactericidal. These authors were, however, mainly concerned with the possible value of the antibiotics for clinical use.

The work on pleuropneumonia-like organisms referred to above was mainly carried out using solid or semi-solid agar culture media. *Ast. bovis* does not grow regularly or well on solid media, and is commonly cultivated in serum broth. In the experiments here described, Bennett's (1932) broth, consisting of ox heart infusion with 2 per cent peptone, and with 10 per cent sterile unheated horse serum added, was used throughout. In this medium *Ast. bovis* gives consistently good growth after 48 hours incubation.

Technique

In general, the selected bacteriostatic substances were dissolved in broth at a given concentration and graded dilutions were made in broth from this. The dilutions were tubed in 4 c.c. amounts and autoclaved at 10 lb. for

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20 minutes. Subsequently 0.5 c.c. of sterile unheated horse serum was added to these tubes, followed by 0.5 c.c. of culture dilution.

In the initial sorting tests, however, culture and serum together was added to 4.5 c.c. volumes of broth containing bacteriostatic substance by the simple expedient of mixing 1 c.c. of undiluted culture with 40 c.c. of sterile serum, and then adding 0.5 c.c. of this serum culture mixture to each tube.

At first crystal violet was dissolved directly in broth and dilutions made from this, but it was found that the initial broth solution deposited rapidly and irreversibly, and a procedure was adopted of keeping a 1/1,000 solution of crystal violet in distilled water, and making dilutions from this in broth as required.

Apart from initial sorting tests, the cultures of *Ast. bovis* were tested in tenfold dilutions as described by Priestley (1951) for dilution counts. In the case of the contaminating organisms which were also used, two or three suitable tenfold dilutions were selected for test. This enabled the critical concentration of any particular substance or combination of substances to be found more satisfactorily than a single and relatively heavy inoculum would do.

All tests were read daily. The duration of incubation was 7 to 10 days for *Ast. bovis*, and 4 to 5 days for contaminants. Adequate control tubes not containing bacteriostatic substances were always included. In the early tests, films stained by stevenel blue (Priestley and Mehdi, 1944; Priestley, 1951) from tubes of *Ast. bovis* taken at random were examined. In the same way, random tubes from the early tests with contaminants were taken and films stained by gram stain were examined. Later the examination of stained films from tubes of contaminants was abandoned, since a positive growth indicated an unsatisfactory level of bacteriostatic substance in any case. When final tests were done on the selected combinations of bacteriostatic substances with various strains of *Ast. bovis*, the highest dilutions of cultures showing growth on any particular day were taken and films stained by stevenel blue were examined.

Organisms Used

For most of the experiments the "F" strain of *Ast. bovis* was used; this was an attenuated strain maintained for the production of culture vaccine. In addition, and in particular for later tests, a number of available strains, ranging from freshly-isolated virulent ones to old attenuated strains, were tested.

The contaminating organisms used to judge the effectiveness of the bacteriostatic substances were as follows:—

1. Morphologically a staphylococcus; white colonies formed on agar medium. Obtained from a contaminated bottle of culture vaccine.
2. As No. 1. Obtained from a contaminated blood agar plate.
3. Morphologically a stumpy gram-negative bacillus. Obtained from a contaminated tube in an early experiment.
4. Morphologically a long, slender bacillus staining feebly gram-positive. Obtained from a contaminated bottle of culture vaccine.
5. As No. 4. Obtained from a contaminated blood agar plate.

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6. Streptococcus viridans type, designated p.38. Obtained from my sputum by culture in serum broth containing thallium acetate, and subsequent plating on blood agar.

7. *E. coli* of human fæcal origin. Obtained from the Sudan Medical Service.

8. A strain of *Pasteurella septica* maintained for the production of hæmorrhagic septicæmia vaccine.

Of the above, Nos. 2, 4, 5, 6, 7, and 8 were used as a set in most of the experiments.

When it was subsequently found that streptococcal contaminants were very troublesome, a number of these organisms were gathered and used. Those designated "E.O." were obtained during attempts to isolate *Ast. bovis* from bovine lung samples as described later. Streptococci used were:—

E.O. 43.—Chain formation. Slight hazy hæmolysis.

E.O. 56.—Chain formation. alpha hæmolysis.

E.O. A15.—Short chains. No hæmolysis.

Stack "H."—Chain formation. Strong beta hæmolysis. A strain of human origin, obtained from the Sudan Medical Service.

Hæmolysis was tested on 10 per cent ox blood agar plates, after 48 hours incubation, followed by 12 hours in the ice chest.

Bacteriostatic Substances Tested

The necessity for using a clear liquid medium means that bacteriostatic substances must be soluble at effective concentration, and must give a minimum of colouration. Preferably they should be stable in broth on autoclaving, and during storage.

Penicillin is not stable, but is otherwise eminently satisfactory; it is therefore dealt with separately later.

Sodium azide, which had been tried by Edward (1947) and by Smith, Morton, and Leberman (1950), was not available, and would appear to offer no great advantage over thallium acetate. Crystal violet was used in preference to gentian violet because the latter is usually a mixture containing crystal violet among other things. Cetavlon, proprietary detergent which is cetyl-tri-methyl-ammonium bromide, was noted by Harper and Cawston (1945) as being useful in the control of *Proteus vulgaris* in plate cultures from mixed wound infections. Boracic acid was reported by Chodkowski (1950) as being of value with potassium tellurite for the isolation of *Str. agalactiæ*. A range of sulphonamides has been included in order to select the most suitable, and with the intention, never fully realised, of controlling streptococcal contaminants. Diphenasone, which is $4:4^1$ diamino-diphenyl-sulphone, and is also known as Udolac, was included as a sulphonamide. Urethane, which is ethyl carbamate, was tried because of the reputed additive effect with sulphonamides.

The selection of substances for initial test was governed by the likelihood of good solubility in and lack of unsuitable interaction with the medium used,

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and finally to a great extent by ready availability. A full list is given in the results of the initial sorting tests.

Initial Sorting Tests with the "F" Strain of *Ast. bovis*

Twenty-three bacteriostatic substances thought to be sufficiently stable under the conditions of test were tried, using the "F" strain of *Ast. bovis*. Culture and serum were added simultaneously to the broth as described earlier.

Alternate fivefold and tenfold dilutions of bacteriostatic substance were made from the initial concentration given. The results were as follows:—

Substance	Solubility in broth	Concentrations tested	Result
Thallium acetate ...	Soluble 1/1,000	1/100 to 1/5,000	
Sulphamezathine ...	Soluble 1/1,000	1/100 to 1/50,000	
Sulphanilamide ...	Soluble 1/1,000	1/1,000 to 1/50,000	
Sulphacetamide ...	Freely soluble	1/100 to 1/50,000	
Sulphapyridine ...	Soluble 1/1,000	1/100 to 1/5,000	All showed good growth in all concentrations
Sulphaguanidine ...	Soluble 1/1,000	1/100 to 1/10,000	
Diphenasone	Insoluble 1/10,000	1/100 to 1/10,000	
Urethane	Freely soluble	1/100 to 1/5,000	
Iodine	Soluble 1/1,000	1/1,000 to 1/10,000	
Thionin blue	Soluble 1/5,000	1/5,000 to 1/50,000	
Sulphathiazole	Soluble 1/1,000	1/100 to 1/10,000	All showed good growth in all except the initial concentration
Boracic acid	Freely soluble	1/100 to 1/10,000	
Basic fuchsin	Soluble 1/5,000	1/5,000 to 1/50,000	
Crystal violet	Soluble 1/5,000	1/5,000 to 1/500,000	All showed good growth in all except the first two concentrations
Trypan blue	Soluble 1/50,000	1/5,000 to 1/500,000	
Sodium taurocholate ...	Soluble 1/100	1/100 to 1/5,000	Showed growth in the last concentration only

Substance	Solubility in broth	Concentrations tested	Result
Cetavlon	Soluble	1/100 to 1/10,000	
Sodium desoxycholate ...	Soluble	1/100 to 1/1,000	
Sodium selenite ...	Insoluble	1/100 to 1/50,000	No growth in any concentration
Proflavine	Soluble	1/100 to 1/10,000	
Brilliant green	Opalescent	1/5,000 to 1/50,000	
Malachite green ...	Soluble	1/5,000 to 1/50,000	
Potassium tellurite ...	Opaque	1/1,000 to 1/10,000	Completely opaque in all concentrations

From these results the last eight substances listed were eliminated.

In the experiment recorded above, tubes showing growth of *Ast. bovis* from some of the tests were further examined by dilution counts, after 3 and 5 days' incubation, together with control tubes, with the intention of determining any bactericidal as distinct from bacteriostatic action. The results did not add anything useful to the information already available, and this line was therefore not pursued.

Further Sorting Tests Using Dilutions of the "F" Strain of *Ast. bovis* and of Contaminants

The remaining fifteen bacteriostatic substances were further tested using tenfold dilutions of *Ast. bovis* at from 1 in 10^4 to 1 in 10^{10}. At the same time they were tried for activity against six contaminating organisms, using two dilutions of culture only, namely, 1 in 10^5 and 1 in 10^7.

The dilutions of *Ast. bovis* were obtained in the following manner:—

0.5 c.c. of a 48-hour-old serum broth culture was added to 4.5 c.c. of plain broth and 0.4 c.c. of this added to 40 c.c. of plain broth. This was used for sowing by serial transfer of 0.5 c.c. through a series of each concentration of bacteriostatic substance in 4.5 c.c. volumes of broth containing 0.5 c.c. of serum. Controls were sown at the same time.

The dilutions of the contaminants were obtained by transferring 0.5 c.c. of a 48-hour-old serum broth culture serially twice through 4.5 c.c. amounts of plain broth, then transferring 0.4 c.c. of this serially twice through 40 c.c. volumes of plain broth, giving two bulk dilutions of 1 in 10^4 and 1 in 10^6 respectively. Each of these bulk dilutions was sown in 0.5 c.c. amount to each concentration of each bacteriostatic substance. In the case of the contaminants no attempt was made to obtain accurate dilutions, and pipettes were not always changed between dilutions. Controls were sown at the same time.

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The concentrations of the bacteriostatic substances used are set out in Table I—see below.

Growth of *Ast. bovis* in this test was unaffected except in four cases:—

Iodine at 1/1,000 suppressed growth in all culture dilutions;

Diphenasone at 1/1,000 and thionin blue at 1/5,000 suppressed growth in all except the first culture dilution;

TABLE 1

SORTING TESTS WITH DILUTIONS OF CONTAMINANTS, USING STABLE BACTERIOSTATIC SUBSTANCES

Bacteriostatic substances		Dilutions of Contaminants.											
		2		4		5		6		7		8	
		Staph.		Bacill.		Bacill.		Strep.		E.coli.		Past.	
Name	Consc.	10^5	10^7	10^5	10^7	10^5	10^7	10^5	10^7	10^5	10^7	10^5	10^7
Thallium acetate	1/1000	-5	-5	-5	-5	-5	-5	+2		p3	-5	p5	+5
	1/5000	-5	-5	p3	p5	p2	p5	+2	+3	+1	+1	+1	+2
	1/10,000	+5	+5	+3	+5	+2	+3	+2	+3	+1	+1	+1	+2
Sulphamezathine	1/1000	+4	-5	p4	p4	-5	-5	-5	-5	p3	p3	+2	+2
	1/5000	p3	-5	p2	p3	-5	-5	-5	-5	p1	p2	+2	+2
	1/10,000	+4	p2	p3	p4	-5	-5	-5	-5	+3	+4	+2	+2
Sulphanilamide.	1/1000	p5	p5	p3	p3	-5	-5	p4	-5	p1	p2	+2	+2
	1/5000	p4	-5	p2	p4	-5	-5	-5	-5	+3	+3	+2	+2
	1/10,000	+2	-5	p2		p4	p5	p4	p5	+2	+2	+2	+2
Sulphetamide	1/1000	+3	+4	p3	p4	-5	-5	p4	-5	+2	+3	+2	+2
	1/5000	+3	-5	p2	p3	p3	p3	+4	p4	+2	+2	+2	+2
	1/10,000	+2	+4	p2	p4	p4	p4	+4	+4	+1	+2	+2	+2
Controls		+2	+2	+2	+2	+2	+3	+2	+2	+1	+1	+1	+1
Sulphapyridine	1/1000	+2	+3	-5	-5	-5	p2	-5	-5	p1	p1	+2	+2
	1/5000	+2	+3	-5	-5	p2	-5	-5	-5	p1	p1	+2	+3
	1/10,000	+2	+2	-5	-5	p2	p2	-5	-5	p1	p1	p2	+3
Sulphathiazole	1/1000	+2	p2	-5	-5	p3	p5	-5	-5	p4	-5	-5	-5
	1/5000	+2	p2	-5	-5	p2	p2	-5	-5	p1	p1	p2	p1
	1/10,000	+2	p2	-5	-5	p1	-5	-5	-5	p1	p2	p2	p2
Sulphaguanidine	1/1000	+2	+3	-5	-5	p2	-5	-5	-5	+2	p3	+2	p3
	1/5000	+2	+2	-5	-5	p4	-5	-5	-5	+2	+2	+2	+3
	1/10,000	+2	+2	p3	-5	-5	-5	-5	-5	+2	+2	+2	+2
Diphenasone	1/1000	+2	+3	p4	-5	p2	-5	-5	-5	+4	p2	p1	-5
	1/5000	+4	+2	-5	-5	-5	-5	-5	-5	+2	+2	+2	+2
	1/10,000	+2	+2	-5	-5	-5	-5	-5	-5	+2	+2	+2	+2
Urethane	1/100	+1	+2	+1	+2	+1	+2	+1	+2	+1	+2	+1	+2
	1/500	+1	+2	+1	+2	+1	+2	+1	+2	+1	+1	+1	+1
	1/1000	+1	+2	+1	+2	+1	+2	+1	+2	+1	+1	+1	+1
Boracic acid	1/1000	+2	+2	+4	+3	+2	+3	+2	+2	+1	+1	+1	+4
	1/5000	+1	+1	+1	+1	+1	+1	+2	+2	+1	+1	+1	+1
	1/10,000	+1	+1	+2	+1	+1	+1	+1	+2	+1	+1	+1	+1
Controls		+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1
Iodine	1/1000												
	1/5000												
	1/10,000												
Crystal violet	1/50,000	-5	-5	-5	-5	-5	-5	+5	-5	+1	+1	-5	-5
	1/100,000	-5	-5	-5	-5	-5	-5	+3	+2	+1	+3	+3	+3
	1/500,000	-5	-5	-5	-5	-5	-5	+3	+5	+1	+1	+1	+3
Trypan blue	1/50,000	+3	-5	+3	+3	+3	+5	+3	p3	+1	+1	+1	+1
	1/100,000	+3	-5	+3	+3	+3	+3	+3	+4	+1	+1	+1	+4
	1/500,000	+3	+3	+1	+3	+3	+3	+3	+4	+1	+1	+1	+3
Basic fuchsin	1/25000	-5	-5	+5	-5	-5	-5	+5	+4	+1	+1	+1	+3
	1/50,000	-5	-5	+3	+3	+3	+3	+3	+5	+1	+1	+3	+3
	1/100,000	+3	+3	+3	+3	+3	+3	+3	+4	+1	+1	+1	+3
Thionin blue	1/5000	+1	+3	+3	+3	+3	+3	+3	+3	+1	+1	p1	p3
	1/25,000	+1	+3	+3	+3	+3	+3	+3	+3	+1	+1	+1	+1
	1/50,000	+1	+3	+3	+3	+3	+3	+3	+3	+1	+1	+1	+1
Controls		+1	+5	+3	+3	+3	+5	+3	+4	+1	+1	+1	+1

Crystal violet at 1/50,000 delayed growth considerably in all culture dilutions.

The results with the contaminants are given in Table I, which also gives

the concentrations of the bacteriostatic substances used with *Ast. bovis*. Iodine was not tested against contaminants at this stage.

From these results, trypan blue, thionin blue, and basic fuchsin were eliminated owing to poor control of contaminants, and to density of colour. Boracic acid and urethane also appeared to be unsatisfactory, and diphenasone has the disadvantage of very poor solubility. These substances, together with some of the sulphonamides and with iodine, were later tested against contaminative streptococci.

Tests with Combinations of Bacteriostatic Substances

In view of the results so far obtained, various combinations of the following five substances were tried: thallium acetate, crystal violet, sulphamezathine, sulphathiazole, and urethane. It was hoped that the addition of a suitable sulphonamide would both control streptococci and help to control gram-negative bacilli. Urethane was included to see whether any additive effect would justify its use.

Double and triple combinations were tried, the tests being put up with dilutions of cultures as described before, but the dilutions of the contaminants were lowered to 1 in 10^3 and 1 in 10^5. The "F" strain of *Ast. bovis* was again used. The results are given in Table II.

From these effects, urethane did not appear to exert any useful effect and was discarded. No sharp differences were shown between the two sulphonamides. The combination finally adopted for further test was: 1/1,000 thallium acetate, 1/5,000 sulphamezathine, and 1/1,000,000 crystal violet. This mixture incorporated in serum broth was designated "Medium 'A.'"

It may be noted that McKenzie (1941) devised a selective medium for the isolation of *Str. agalactiæ* which consisted of lemco glucose broth containing 1/1,000 thallium acetate and 1/500,000 crystal violet. Thallium acetate is particularly useful in controlling sporing bacilli and some fungi.

Tests Using Different Strains of *Ast. bovis*

During the course of the preceding experiments, opportunity was taken to check the effect of thallium acetate, sulphamezathine, sulphanilamide, and crystal violet all separately, at concentrations already found not to affect the growth of the "F" strain of *Ast. bovis*, on four other attenuated strains of Sudan origin, and a vaccine strain from East Africa. The tests were put up in the manner described for initial sorting tests, and full growth was always obtained.

Later, dilutions of from 1 in 10^1 to 1 in 10^{10} of seven different strains of *Ast. bovis* were tested in Medium "A." The strains used included freshly-isolated virulent ones, a first and a twentieth subculture from a freeze-dried culture, and old attenuated strains, and full growth was obtained in all dilutions of all cultures.

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Trials with Medium "A"

Opportunity arose to test Medium "A" during an outbreak of contagious bovine pleuropneumonia in the field. One-ounce screw-capped bottles containing 10 c.c. of the medium were sent to the slaughterhouse, and suspected lung tissue placed in them without any sterile precautions. The seeded bottles were despatched to the laboratory by air transport at intervals of 3 to 4 days, then incubated, and films stained by stevenel blue were examined.

TABLE 2

TESTS WITH DILUTIONS OF AST. BOVIS AND OF CONTAMINANTS USING COMBINATIONS OF STABLE BACTERIOSTATIC SUBSTANCES.

Bacteriostatic substances Combinations	Concs.	Dilutions of Ast. bovis 10⁴	10⁵	10⁶	10⁷	10⁸	10⁹	10¹⁰	Dilutions of Contaminants. 2 Staph 10³	10⁵	4 Bacill 10³	10⁵	5 Bacill 10³	10⁵	6 Strep. 10³	10⁵	7 E.coli 10³	10⁵	8 Past. 10³	10⁵
Thallium acetate Crystal Violet	1/1000 1/500,000	+2	+2	+3	+4	+4	+5	-7		-5		-5		-5		+1		p2		-5
Thallium acetate Sulphamezathine	1/1000 1/5000	+2	+2	+3	+4	+4	+4	+5		-5		-5		-5		-5		-5		-5
Sulphamezathine Crystal violet	1/5000 1/500,000	+2	+2	+3	+3	+4	+5	+5		-5		-5		-5		-5		+5		p2
Controls		+2	+2	+3	+3	+4	+4	-7		+1		+2		+2		+2		+1		+1
Thallium acetate Sulphamezathine	1/1000 1/5000	+2	+3	+3	+3	+4	+4	+5	p2	-5	-5	-5	-5	-5	-5	-5	p1	p3	p1	p5
Thallium acetate Sulphathiazole	1/1000 1/5000	+2	+2	+3	+3	+4	+4	-6	p1	p2	-5	-5	-5	-5	-5	-5	p4	p3	p1	-5
Sulphamezathine Crystal violet	1/5000 1/500,000	+2	+3	+3	+3	+4	+4	+5	p4	-5	-5	-5	-5	-5	-5	-5	+2	p3	p1	p3
Sulphathiazole Crystal violet	1/2000 1/500,000	+2	+3	+3	+4	+4	+5	+5	p2	-5	-5	-5	-5	-5	-5	-5	p1	p3	p1	p3
Thallium acetate Sulphamezathine Crystal violet	1/1000 1/5000 1/1000,000	+2	+2	+3	+3	+4	+4	-6	-5	-5	-5	-5	-5	-5	-5	-5	p1	p3	p1	p5
Thallium acetate Sulphathiazole Crystal violet	1/1000 1/5000 1/1000,000	+3	+2	+3	+4	+4	+4	+5	-5	-5	-5	-5	-5	-5	-5	-5	p1	-5	p1	-5
Controls		+2	+2	+3	+3	+4	+4	-6	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1
Thallium acetate Sulphamezathine	1/1000 1/5000	+2	+2	+3	+3	+3	+4	-9	p4	-5	-5	-5	-5	-5	-5	-5	p1	-5	p1	-5
Thallium acetate Sulphamezathine Crystal violet	1/1000 1/5000 1/500,000	+2	+2	+3	+3	+4	+5	-9	-5	-5	-5	-5	-5	-5	-5	-5	p1	-5	p1	-5
Thallium acetate Sulphamezathine Crystal violet	1/1000 1/5000 1/1000,000	+2	+2	+2	+3	+3	+4	+4	-5	-5	-5	-5	-5	-5	-5	-5	p1	-5	p1	-5
Thallium acetate Sulphamezathine Urethane	1/1000 1/5000 1/500	+2	+2	+3	+3	+3	+3	+4	p4	-5	-5	-5	-5	-5	-5	-5	p1	-5	p1	-5
Thallium acetate Sulphamezathine Urethane	1/1000 1/5000 1/1000	+2	+2	+3	+3	+3	+4	-9	p4	-5	-5	-5	-5	-5	-5	-5	p1	-5	p1	-5
Controls		+2	+2	+2	+3	+3	+3	-9	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1

Of 147 specimens (animals), 71 showed growth of *Ast. bovis*, of which 42 were pure cultures. Only 15 bottles remained sterile after incubation, and 121 showed streptococci as the only contaminating organism. This test was probably unduly severe in view of the necessarily rough-and-ready way in which the bottles were seeded, and of the very high incidence of aerial contamination normally experienced in the Sudan. Even so, the failure to control streptococcal contaminants was considered unsatisfactory, and an attempt was made to deal with this.

Special Tests with Streptococcal Contaminants

The streptococcal contaminants earlier described as E.O. streptococci, together with the human hæmolytic strain "Stack 'H,'" and the streptococcus p.38 (Contaminant No. 6), used in previous tests, were all used.

All the sulphonamides including diphenasone, together with sodium taurocholate, sodium desoxycholate, and iodine, were retested against the streptococci. Extra concentrations of 1/100 and 1/500 were included in the case of sulphamezathine, sulphanilamide, and sulphacetamide.

In addition, to check whether the sulphonamides were either broken down during sterilisation in broth, or were inactivated in the presence of horse serum, a further experiment was put up. The same three sulphonamides mentioned above were sterilised in broth as for all previous experiments, but no serum was added. They were also sterilised separately in distilled water and added to sterile broth with serum.

No effect attributable to either the presence of horse serum, or to the sterilisation of the sulphonamides in broth, could be demonstrated. No definite suppression of the streptococcal contaminants from bovine lung tissue was obtained by any of the substances tested, with cultures used at dilutions of 1 in 10^3 and 1 in 10^5. The streptococcus of human origin was partially, but not entirely, suppressed by the sulphonamides, even with these at a concentration of 1/100. The original streptococcus p.38 was sensitive to all the sulphonamides.

No further attempt was made, and Medium "A" remained unchanged.

Experiments with Penicillin

Technique

Apart from the final tests, all experiments were done in the same way as described for those with other bacteriostatic substances, except that the broth was sterilised in volumes sufficient for the number of tubes of 4 c.c. or 4.5 c.c. required, and calculated to allow suitable serial dilutions of the penicillin to be made in bulk. Following this, soluble crystalline "G" penicillin in phials of 100,000 or 200,000 units was dissolved in a suitable initial volume of the sterile broth, and dilutions made in the prepared bulks of broth. These were then tubed aseptically in sterile tubes. Serum was added to the tubes of broth already containing the penicillin.

In the final tests and trials, however, penicillin and serum were added simultaneously to the broth as described later.

Organisms

These were the same as those used for all previous experiments.

Initial Tests with *Ast. bovis*

Five strains of *Ast. bovis* of Sudan origin and two strains from East Africa were sown in serum broth containing penicillin at from 10,000 to 10 units per c.c., by the rough method described earlier. Full growth was obtained in all cases; the organism is apparently unaffected by very high concentrations of penicillin.

Preliminary Test with Contaminants

The eight contaminants were used at a final dilution of 1 in 10^3, obtained by diluting 0.5 c.c. of a 48-hour-old serum broth culture in 4.5 c.c. of broth,

then adding 4 c.c. of this to 40 c.c. of broth, and sowing 0.5 c.c. of this bulk dilution to tubes of 4.5 c.c. serum broth containing penicillin at concentrations of 2,000 and 500 units per c.c.

Contaminant No. 7 (*E. coli*) showed growth in 500 units per c.c. at 2 days incubation, but was suppressed with penicillin at 2,000 units per c.c. All other organisms were completely suppressed, all the tubes remaining sterile after 4 days incubation, with controls tubes showing full growth at 1 or 2 days incubation.

Tests with Dilutions of the "F" Strain of *Ast. bovis* and of Contaminants

These tests were carried out as described for previous tests with dilutions of organisms. Penicillin was used at 500, 100, and 10 units per c.c., and *Ast. bovis* culture at dilutions of from 1 in 10^4 to 1 in 10^{10}. The cultures of contaminants were tested at dilutions of from 1 in 10^4 to 1 in 10^8, and the same set of the six contaminants as were used in the tests with other bacteriostatic substances were again used.

There appeared to be some inhibition of *Ast. bovis* at extreme dilutions by penicillin at a concentration of 500 units per c.c. (this was not confirmed by later tests); otherwise growth of this organism was normal.

All the dilutions of all the contaminants except *E. coli* showed no growth after prolonged incubation. *E. coli* was completely suppressed by penicillin at 500 units per c.c., but grew in all culture dilutions with penicillin at 10 units per c.c. This organism is known to be refractory to penicillin because of the production of penicillinase.

It is apparent that the lower level of penicillin would be sufficient to control most contaminants.

Dilutions of Five Strains of *Ast. bovis*, adding Penicillin and Serum Simultaneously

Five of the strains of *Ast. bovis* used for the corresponding test with Medium "A" were again used in this experiment, in dilutions of from 1 in 10^3 to 1 in 10^{10}. Penicillin was tested at 500, 100, and 10 units per c.c. In this case, however, the penicillin was dissolved in 1 or 2 c.c. of broth and added to a known bulk of serum from which 0.5 c.c. amounts were added to tubes of broth. A separate bulk of serum was used for each concentration of penicillin.

The results showed no obvious suppression of any dilution of any strain of *Ast. bovis* at 500 units per c.c. or less of penicillin.

Trial with Penicillin

The outbreak of contagious bovine pleuropneumonia which was utilised for a trial of Medium "A" as already described also served for a trial of penicillin. Serum broth containing penicillin at a concentration of 100 units per c.c. was prepared by sterilising 9 c.c. volumes of broth in one-ounce screw-

capped bottles, to each of which was subsequently added 1 c.c. of serum containing 1,000 units of penicillin. The bottles of medium were prepared immediately before dispatch from the laboratory, but variable delays of several days necessarily occurred both before and after seeding.

Of 125 specimens (animals), 17 were positive for growth of *Ast. bovis,* and of these 15 were pure cultures. Only 16 bottles remained sterile after incubation. The type of contamination varied but did not include many streptococci.

In this series of 125 specimens, 73 were seeded at the same time into both Medium "A" and into the penicillin serum broth, and comparative results were as follows:—

	Samples showing growth of *Ast. bovis*	Samples not showing growth of *Ast. bovis*	Samples showing contamination
Medium "A"	37 (27 pure)	36 (5 sterile)	41
Penicillin serum broth ...	16 (14 pure)	57 (13 sterile)	46
Both media simultaneously	13 (10 pure)	33 (5 sterile)	31
Totals	40 (31 pure)	33 (13 sterile)	51

These results reflect the instability of penicillin.

In the laboratory, as a control measure in experimental culture work with *Ast. bovis,* penicillin has proved successful. It is convenient and easy to add to media and test material at any stage of culture work, and is cheap. It has been used to purify culture tests of *Ast. bovis* which have become contaminated following repeated manipulation. A level of 10 to 20 units per c.c. is suitable.

A rough trial of growing *Ast. bovis* as for culture vaccine production, but using bulk cultures with loose screw caps in place of cotton-wool plugs was tried. Bulks of 425 c.c. of serum broth containing 500, 100, 50, and 10 units of penicillin per c.c. were used for initial seeding and incubation, and subsequently the grown cultures were distributed in sterile 50 c.c. and 100 c.c. vaccine bottles by pouring. The small vaccine bottles were immediately corked and further incubated; the total time was extended to 23 days. All the large bulks remained pure, and only one small vaccine bottle showed contamination.

The test was unsatisfactory because no controls were included, but in routine culture vaccine production with aseptic precautions but without bacteriostatic substances contamination of a number of vaccine bottles is a very frequent occurrence.

Discussion

The combination of bacteriostatic substances used in Medium "A" gives a stable medium which can be prepared, sterilised, and stored in quantity against possible requirements. It would appear to be suitable for the isolation of *Ast. bovis* from field specimens.

Penicillin has the disadvantage of instability, and it has to be added to

media after sterilisation, although in the case of the serum broth this can be done at the same time that the serum is added. The action of penicillin against contaminants will disappear in a relatively short time, so that it would not be so useful for field specimens.

Penicillin requires no preparation, and the range of tolerance by *Ast. bovis* is extremely wide, so that a much greater control of contaminants initially is possible than by other means.

In relation to contagious bovine pleuropneumonia culture vaccine preparation on a large scale, where contamination is always a problem to be considered, the use of any bacteriostatic substance necessitates first a careful examination of the effect, if any, on the virulence and on the immunising power in cattle of the vaccine strains of *Ast. bovis* used. This has been considered worth while in the case of penicillin, and such an investigation is being carried out.

The Medium "A" combination contains thallium acetate which is known to be toxic in animals. The dose of thallium acetate per dose of vaccine would be 1 to 2 milligrammes subcutaneously.

Summary

A combination of thallium acetate 1/1,000, sulphamezathine 1/5,000, and crystal violet 1/1,000,000 in Bennett's (1932) broth was found to allow full growth of *Ast. bovis* in extreme dilutions, and to suppress other organisms chosen as representative of casual contaminants. Streptococcal contaminants occurring grossly were not satisfactorily controlled, and attempts to overcome this by using different sulphonamides in very high concentrations were not successful.

In Bennett's (1932) broth, penicillin at 500 units per c.c. or less is tolerated entirely by *Ast. bovis* in extreme dilutions, and as much as 10,000 units of penicillin per c.c. allowed full growth of this organism with an undiluted inoculum. Contaminants were easily controlled while the penicillin lasted with the exception among the organisms tested of *E. coli*.

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ON THE BASE EXCESS, THE ALKALI ALKALINITY, THE ALKALINE EARTH ALKALINITY AND THE MINERAL RATIOS IN GRASS AND HAY WITH REFERENCE TO GRASS TETANY AND OTHER DISORDERS IN CATTLE

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1. Introduction

IT is generally accepted that cows' urine should be alkaline to litmus. As a matter of fact, this condition prevails in the urine of cows grazing on good pastures. Acidity of the urine is considered as undesirable. It may result, *inter alia*, from loss of appetite or from feeding with mineral acid silage (Brouwer, 1935; Brouwer and Dijkstra, 1936).

Some time ago, however, we observed an acid reaction of the urine from cows fed on grass only and not suffering from loss of appetite. A comparative study was made of the protein content and mineral composition (K, Na, Ca, Mg, Cl, S, P) of nine samples of such grass,* and of hay and grass from other pastures. For this comparison we made use of three sets of analyses published

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respectively by Maschhaupt, *et al.* (1922) (hay from pastures in the province of Friesland), by Sjollema (1931) (grass from pastures having induced grass tetany), and by Hart (1944/45) (normal artificially dried grass). The figures adopted from the latter two authors are the averages of the results of several single analyses.

2. The Acid-base Diagram

In the first instance we asked ourselves whether the urine acidity could be explained by a negative base-balance in the grass. For studying the acid-base-equilibrium in simple feeding stuffs and in complex rations we use a diagrammatic representation, namely, the *acid-base-diagram* (Brouwer, 1951 [2]). As far as we are aware, this method has not been applied yet by any other author.

In this diagram (Fig. 1) the number of equivalents of base-forming elements per kg. of dry matter (total base = Na + K + Ca + Mg) has been plotted out against the number of milliæquivalents of acid-forming elements (total acid = Cl + S + P). One gramatom of P may be valued either at 3 or at $1\frac{1}{2}$ æquivalents. Throughout this article, however, we have used 3 as conversion factor. If $1\frac{1}{2}$ had been adopted as factor it would not have affected our conclusions materially.

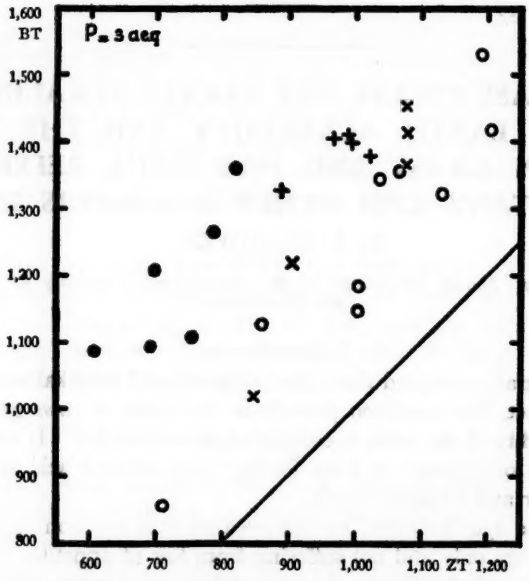

FIG. 1. *Acid-base-diagram* (*P = 3 æquivalents*)

Abscissa: ZT = total acid = Cl + P + S (milliæquivalents per kg. of dry matter).
Ordinate: BT = total base = K + Na + Ca + Mg (milliæquivalents per kg. of dry matter).
Dots: hay samples.
Circles: grass from pastures inducing urine acidity.
Plus symbols: ordinary artificially-dried grass.
Multiplication symbols: grass from grass tetany pastures.

Figure 1 gives direct information of four factors designated BT, ZT, TT and VT respectively :—

$$BT = K + Na + Ca + Mg = \text{total base},$$
$$ZT = Cl + S + P = \text{total acid},$$
$$TT = BT + ZT = (K + Na + Ca + Mg) + (Cl + S + P),$$
$$VT = BT - ZT = (K + Na + Ca + Mg) - (Cl + S + P).$$

ZT and BT have been plotted out on the abscissa and the ordinate; they need no further comment.

As to TT, it is constant on every line that cuts off equal positive (or equal negative) portions from both co-ordinates. Lines parallel to these, but further away from the left-hand bottom corner and closer to the right-hand top corner of the chart, would notify higher TT values.

Furthermore, VT, the base-excess, is constant on any line running parallel to the slanting line in the chart. On this slanting line itself VT is zero. In the section below and on the right-hand side of the slanting line it is negative; on the opposite side it is positive.

In scrutinising the graph it may be seen that in all samples the base-balance VT was positive, and without wide variations. In the samples from the grass which caused urine acidity, represented by circles, VT was certainly lower than in the others, but still distinctly positive. Therefore, the acidity of the urine cannot be explained by a negative base-balance in the grass. Besides, a calculation brought out that the quantity of organic acids in the urine was far too small to yield a satisfactory explanation.

As for TT, in the grass inducing urine acidity this value fluctuated a great deal. Its range includes the artificially dried grass (plus symbols) and the grass from tetany pastures (multiplication symbols). Therefore, the TT values present no new aspect of the problem.

3. The Alkalinity Diagram

In numerous researches on the mineral metabolism of ruminants it has been shown that excesses of Ca, Mg and P in the food leave the body mainly in the fæces. The elements K, Na, Cl and S are far better absorbed in the intestinal tract than those just quoted, and surpluses of the latter are mainly removed from the body by the kidneys. It is therefore obvious that the acid-base-equilibrium of blood and urine is much more affected by the second range of elements than by the first one.

On these considerations we were led to calculate the base-excess of the two mentioned ranges of minerals separately. Following the example of some Hungarian investigators (Hutyra, *et al.*, 1938; Marek, *et al.*, 1931; Wellmann, 1931), we call these excesses the *alkali alkalinity* (AA) and the *alkaline earth alkalinity* (EA) (Brouwer, *et al.*, 1951). As far as we know, their physiological importance has never been profoundly studied. A graph in which these two quantities are

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plotted (Fig. 2) provides direct information of the following four quantities:—

$$AA = Na + K - Cl - S,$$
$$EA = Ca + Mg - P,$$
$$TA = AA + EA = (Na + K - Cl - S) + (Ca + Mg - P),$$
$$VA = AA - EA = (Na + K - Cl - S) - (Ca + Mg - P).$$

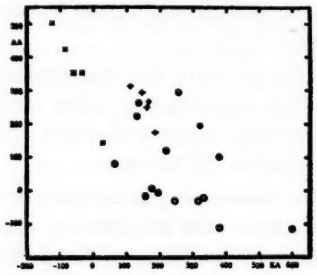

FIG. 2. *Alkalinity-diagram* ($P = 3$ *æquivalents*)

Abscissa: EA = alkaline earth alkalinity = Ca + Mg — P (milliæquivalents per kg. of dry matter).

Ordinate: AA = alkali alkalinity = Na + K — Cl — S (milliæquivalents per kg. of dry matter).

Dots: hay samples.
Circles: grass from pastures inducing urine acidity.
Plus symbols: ordinary artificially-dried grass.
Multiplication symbols: grass from grass tetany pastures.

It is easy to see that TA is identical with the base-excess VT as elucidated in the preceding section. In Fig. 2, TA is constant on lines cutting off equal portions from the co-ordinates. Its value increases in moving from the left-hand bottom corner of the chart to the right-hand top corner.

VA, on the other hand, is constant on lines perpendicular to those just mentioned. Its value is lowest in the right-hand bottom corner and increases while moving from this corner to the left-hand top corner.

The graph shows at once that the AA values of the samples from pastures inducing acid urine (the circles) are, as a rule, much lower than those of the other samples. As a matter of fact, in most cases, AA is even negative. This fact, in conjunction with the consideration that a certain amount of organic acid is also excreted by the kidney, affords a reasonable explanation for the acid reaction of the urine in animals grazing on the affected pastures.

In studying the graph more closely it was impressed upon us that the distribution of the samples showed interesting peculiarities bearing on the health of the animals that ate the relevant grass or hay. In the first instance, we would draw attention to the centre of the chart. It is occupied by symbols representing the artificially dried grass (plus symbols), and by symbols representing most of the hay samples (dots). As far as we know, the relevant grass and hay were not injurious to the health of the animals.

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Contrary to these, the samples scattered over the part of the chart more remote from the centre must nearly all arouse suspicion. This applies in the first place to the grass from the tetany-inducing pastures (multiplication symbols). It is characterised by negative EA values and extremely high AA and VA values, the TA values being intermediate ones. Only one sample of this group shows an intermediate AA value and a slightly positive EA value. This particular sample, however, is not representative of real tetany-inducing grass, because it had been cut two months after the disease had occurred on the pasture concerned.

The two dots below and on the right-hand side of the centre represent hay samples from two rough grazings not protected by dikes. This land suffers from high water levels and is insufficiently fertilised. Especially, the sample plotted out in the right-hand bottom corner is characterised by a high EA value and extremely low AA and VA values. The quality of this hay is considered by practical farmers as very poor. By feeding this kind of hay, bone disease develops if no supplementary food is given. Finally, we draw the attention to the wide fluctuations of VA. The range of fluctuation is between -714, as in the last-mentioned sample, and $+631$, as in the tetany-inducing grass.

Returning to the circles, i.e., the samples of the pastures inducing urine acidity, it will be noticed that they occur below the centre of the graph (low AA values, yet most of them showing normal EA values). These samples, too, must be considered with some suspicion, because the relevant pastures not only give rise to urine acidity, but may also induce alimentary hæmoglobinuria. We are of the opinion that the latter complaint is not directly caused by the low alkali alkalinity (AA) of this grass. Nevertheless, hæmoglobinuria and urine acidity might be associated somehow.

From all these observations, and from several other ones not mentioned here, it occurs to us that, in the alkalinity diagram, AA, EA, TA and VA should occupy a central position with the following tentative limits:—

AA = 100 to 375,
EA = 50 to 325,
TA = 300 to 550,
VA = -150 to $+250$.

4. The Acid Diagram

The conspicuous variations of base-excess and alkalinity studied in the preceding sections must necessarily be due to fluctuations in the proportions of the mineral constituents of the grass. Therefore, the group of the acid-forming elements and the one of the base-forming elements were studied separately. Especially, the *ratios* of the elements proved to be important. Again they can be studied very advantageously from diagrammatic representation.

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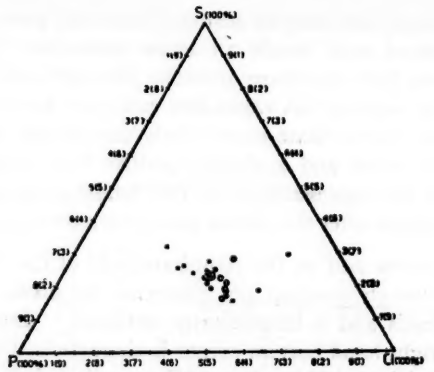

FIG. 3. *Triangular acid-diagram* ($P = 3$ *æquivalents*)
The perpendiculars on the three sides represent in each sample the proportions of Cl, S and P (milliæquivalents per kg. of dry matter), expressed as percentages of their aggregate. These percentages can be read off from the graph with the aid of the scales on the sides of the triangle.

Dots: hay samples.
Circles: grass from pastures inducing urine acidity.
Plus symbols: ordinary artificially-dried grass.
Multiplication symbols: grass from grass tetany pastures.

The proportions of the milliæquivalents of the three acid-forming elements (Cl, S and P), expressed in percentages of their aggregate, have been plotted out in the triangular graph, Fig. 3. We call it the *acid diagram* (Brouwer, 1951 [2]). For every sample these percentages are represented by the three perpendiculars dropped on the sides. It is evident at once that the fluctuations exhibit rather narrow limits. Nevertheless, two dots and one circle bearing upon two hay samples and one grass sample are rather far away from the others. They are characterised by low P values, on account of scanty dressings of phosphate to the pastures. Although this is important from a farming point of view, the acid diagram hardly discloses anything about the causes of the fluctuations in base-excesses and alkalinity.

5. The Base Diagram

Preliminary investigations proved that the ratios of the base-forming elements required special attention. As there are four of them (K, Na, Ca and Mg), a triangular diagrammatic representation is useless, the latter only being suitable for co-ordination of three magnitudes. It is, however, possible to make use of the property of a regular tetrahedron, implying that the sum of the four perpendiculars dropped from arbitrary points within the tetrahedron on the four faces is constant (Brouwer, 1951 [1, 2]). Thus, for every sample, the rates of the four bases, expressed as percentages of their aggregate, can be imagined to be plotted out in a regular tetrahedron. Of this tetrahedron a horizontal and vertical projection can be drawn on a sheet of paper (Fig. 4). The right-hand triangle is the horizontal projection of the tetrahedron resting with one of its faces on the horizontal plane of projection. It has been constituted by so-called central projection from the vertex of the tetrahedron as a centre. The triangle

on the left is the vertical projection. It has been constituted by ordinary orthogonal projection.*

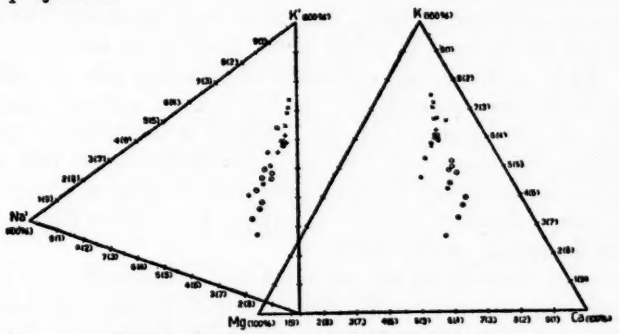

FIG. 4. *Regular tetrahedral base-diagram*
The samples are imaginarily plotted in a regular tetrahedron. The perpendiculars on the four faces represent for each sample the proportions of K, Na, Ca and Mg (milliæquivalents per kg. of dry matter), expressed as percentages of their aggregate. In the vertical projection (in the left-hand triangle) the percentages of K and Na can be read off directly with the aid of the scales on the triangle sides. The right-hand triangle has been obtained by central projection from the vertex *Na* of the tetrahedron as a centre. In this triangle the proportions of K, Ca and Mg can be read off as percentages of their aggregate (K + Ca + Mg), by making use of the scales on the three triangle-sides. To convert them into percentages of the aggregate (K + Na + Ca + Mg) the just-mentioned rates of K, Ca and Mg should be multiplied by $\frac{100}{100 - Na}$

Dots: hay samples.
Circles: grass from pastures inducing urine acidity.
Plus symbols: ordinary artificially-dried grass.
Multiplication symbols: grass from grass tetany pastures.

From Fig. 4, bearing on the samples as the previous graphs, it is evident that the symbols representing the samples are scattered over a wide range. The fluctuations of the ratios, within the group of the base-forming elements, are therefore much wider than those in the group of the acid-forming ones. Obviously, of these fluctuations the one shown by potassium is of paramount importance. The potassium value namely is very low in the hay originating from a rough grazing (represented by a dot) and also in most of the samples taken from pastures inducing urine acidity. On the other hand, it is very high in the grass from the tetany pastures (multiplication symbols). Intermediate are the samples of good hay and artificially dried grass. Obviously, the variations in alkalinity described in one of the preceding sections, essentially find their origin in the fluctuations in the K values.

* It should be noted that the tetrahedron need not necessarily be a regular one. In some other instances we have made use of an equilateral tetrahedron with one pair of opposite edges crossing each other at right-angles. If, in this case, the previously-mentioned pair of opposite edges have a dimension r, it is advantageous to give the four other edges a dimension $\frac{1}{2} r\sqrt{5} = 1.118\ r$. By placing this equilateral tetrahedron in such a way that one of the two edges measuring r is perpendicular to one of the planes of projection and the other perpendicular to the second plane, it follows that both projections are equilateral triangles. In these triangles for every sample there are six perpendiculars, all six retaining a definite import of their own. A full account of various kinds of diagrams for studying the ratios of four magnitudes (a regular tetrahedral graph, an equilateral tetrehedral graph and a square graph) is presented in another paper by the author (Brouwer, 1951, 3). In Fig. 4 we have adhered to the regular tetrahedral graph.

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It is evident that the K values are largely decisive in determining the place of the samples in the diagram. For, with increasing K values, the Na, Ca and Mg values are, all three of them, decreasing, and conversely. It could even be shown that the decrease in values of the latter is not only relative but also absolute. Thus, with two heavy dressings of potassium salt, not only does the K content of the grass increase but the Mg and Ca contents diminish correspondingly. It will be clear that this condition is conducive to the outbreak of grass tetany. Of course, this does not exclude the possibility that other agents are conducive to the disease; protein if present in the grass at a high rate, might be one of them. Recently, Deys, *et al.* (1951), have shown that there are also fluctuations depending on the season.

From other observations we concluded that, occasionally, low Mg contents of the grass may also occur with intermediate K values. In these cases the Ca/Mg ratios are high, but up to now we have not been able to decide whether these low Mg values are primary or whether they result from high Ca contents.

Also the fall in the Na content of the grass caused by excessive dressings with K salts, has practical consequences for farm practice. For on such pastures the animals display a conspicuous craving for kitchen salt which, of course, should be satisfied.

For all these reasons excessively high applications of potassium fertilisers to pastures should be avoided. On the other hand, scanty dressings of potash should be avoided as well, not only for the sake of a luxuriant grass crop, but also with the aim to maintain proper conditions of health for the animals.

6. Summary

In samples of grass and hay of various origins the base-excess, the alkali alkalinity, the alkaline earth alkalinity, the ratios of the base-forming elements and those of the acid-forming elements were studied with the aid of diagrammatic representations (acid-base-diagram, alkalinity-diagram, triangular acid-diagram and regular tetrahedral base-diagram).

Grass from pastures inducing urine acidity in grazing dairy cattle contained, despite the acid urine reaction, a considerable base-excess (K + Na + Ca + Mg — Cl — S — P in milliæquivalents per kg. of dry matter). The alkali alkalinity (K + Na — Cl — S), however, was negative in most of these grass samples and affords a better explanation of urine acidity than the base-excess.

Grass from tetany-inducing pastures was characterised by extremely high alkali alkalinity and extremely low alkaline earth alkalinity (Ca + Mg — P in milliæquivalents per kg. of dry matter). Its base-excess was about normal.

On the other hand, in hay from a rough grazing the condition was quite the opposite: extremely low alkali alkalinity and extremely high alkaline earth alkalinity. Again the base-excess was about normal. This hay induces bone disease if supplied as the only feed.

In normal grass and hay the alkali alkalinity and alkaline earth alkalinity are intermediate and fluctuate between certain limits that have been tentatively described in the text.

By the triangular acid-diagram it is shown that the ratios of the three acid-forming elements (Cl, S and P), as a rule do not fluctuate a great deal although their aggregates may vary considerably. Phosphate deficiency of the soil, however, results in a decrease of P and a relative increase of Cl and S.

Within the group of the base-forming elements (K, Na, Ca and Mg), studied with the aid of the regular tetrahedral base-diagram, the exchanges proved to be much more important than with the acids. The cause of these exchanges must mainly be attributed to potassium. With increasing K values, the contents of Na, Ca and Mg all decrease. This condition is conducive to an outbreak of grass tetany. On the other hand, in hay from a rough grazing and, as a rule, also in grass inducing urine acidity with the animals, the K content was low, the proportions of Na, Ca and Mg having increased. Excessive as well as scanty dressings of potassium salts should be avoided in order to keep the animals in good health.

The fluctuations in alkali alkalinity and in alkaline earth alkalinity described previously proved to be first of all dependent upon the potassium content of the grass.

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EXPERIENCES WITH GENERAL ANÆSTHESIA IN RATS

By MARK SWERDLOW, M.D.(Manc.), Ch.B., B.Sc., D.A.

DURING research on the effects of anæsthesia on the rat adrenal,[1] the author administered anæsthetics to 263 rats. The following observations are based on the experience gained therein.

Experimental Animal

Healthy adult male albino rats of the Wistar strain were used exclusively. The average weight of the rats used was 132.4 gm.; 95 per cent weighed between 110 gm. and 155 gm. (incl.).

Anæsthetic Agents

Ether, chloroform, trichlorethylene, nembutal and pentothal were used. Each was administered to two groups of rats on a dose/weight basis.

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Anæsthetic Technique

With the volatile anæsthetics, induction was carried out with a mask and drop-bottle, and anæsthesia maintained by intermittent addition of small doses using a method which has been described elsewhere.[2] The barbiturates were administered by intermittent intraperitoneal injection. Atropine was not employed as a premedicant drug.

With all anæsthetics, depth of anæsthesia was tested every four minutes by gently squeezing the rat's tail. A certain degree of reflex response to stimulation was taken as the index for adding a maintenance dose of anæsthetic; the degree of response differed for each anæsthetic. In this way a fairly level plane of anæsthesia was maintained with each anæsthetic.

Duration of Anæsthesia

With each anæsthetic two series of rats were submitted to anæsthesia of approximately one and two hours' duration respectively. In one small group of rats, anæsthesia was maintained for five hours with ether; this was not found possible with the other anæsthetics used.

OBSERVATIONS

1. *General.*

It was found, as would be expected, that progressively less anæsthetic was required as time passed, i.e., a positive response to stimulation was obtained at lengthening intervals.

It was noted that frequently smaller rats tolerated anæsthesia better than heavier ones, and recovered earlier from anæsthesia. There were more fatalities among the heavier animals. Dreyer and Walker[3] consider that in warm-blooded animals of the same species, but different weights, dosage should be calculated in relation to body surface. It may be that the use in the present work of body weight rather than surface area in the estimation of anæsthetic dosages accounts in part for this apparently greater tolerance of the smaller rats. Differences in metabolic rate may be a further factor.

2. *Specific.*

ETHER.

This proved to be the safest of the agents investigated, and depth of anæsthesia could most easily be judged. After administration of an overdose, artificial respiration usually proved successful. At the end of anæsthesia the rats recovered quickly and moved around the cage fairly actively.

In some early experiments the possibility of administering ether in measured doses by the intraperitoneal route was investigated. (Intravenous ether has been used to produce anæsthesia in man.) Ether diluted with warm saline was injected intraperitoneally in intermittent doses, and after some experiment the dosage was found whereby anæsthesia could satisfactorily be maintained for any required period of time. However, few of the animals survived for 24 hours after anæsthesia. Post-mortem examination showed the peritoneum to be inflamed and full of bloodstained exudate.

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CHLOROFORM.

This was the second most toxic anæsthetic, and many fatalities occurred. As with trichlorethylene, the commonest cause of death was the sudden increase in concentration which occurred on addition of a supplementary dose of anæsthetic. The frequency of such mishaps was reduced by using the minimal size of maintenance dose. A much grosser movement of tail and limbs than with ether was taken as the indication for adding a supplementary dose of anæsthetic.

With both chloroform and trichlorethylene the advent of respiratory arrest from overdose could be foreseen. The normally rapid but smooth, regular breathing changed to a jerky, slower respiration, which in a few breaths diminished in depth to complete cessation. The heart usually continued beating for a few moments after the respiration had ceased. If artificial respiration was applied promptly before respiration had ceased, successful resuscitation was frequently achieved, particularly after chloroform. If the overdose was not noticed until after cessation of respiration, resuscitation was unlikely to be successful.

After recovery from chloroform anæsthesia the rats behaved in a somewhat drunken fashion for an hour or two.

It is interesting to note that in one early batch of rats which were given an overdose of chloroform and died a short time after one hour's measured anæsthesia, post-mortem examination revealed multiple hæmorrhages in the lungs.

TRICHLORETHYLENE.

This anæsthetic was found to be very toxic in rats, and the greatest number of fatalities occurred in this series. In the early experiments death from overdosage at induction was quite common.

In order to avoid cyanosis (which usually denoted an impending fatality) a very light plane of anæsthesia only had to be maintained. Thus induction was carried out very cautiously with a minimal quantity of the drug. Maintenance doses were not given until testing for depth produced a gross movement of body, tail and limbs. After anæsthesia the rats remained somewhat sleepy and moved around the cage in a much more lethargic manner than either of the two previously described anæsthetic groups.

NEMBUTAL.

The veterinary solution containing 0.065 gm. Nembutal per ml. was diluted with warm normal saline to make a 1 per cent solution.

Nembutal proved the second safest of the anæsthetics used, but had the disadvantages that depth of anæsthesia could not so easily be altered or kept level, and that recovery was much slower than after the volatile anæsthetics. Emergence from anæsthesia was not as clearly defined as with the inhalationary agents. After anæsthesia the animals remained still and sleepy, although moving around the cage when stimulated.

PENTOTHAL.

The 5 per cent solution obtained by dissolving $\frac{1}{2}$ gm. of powder in 10 ml. distilled water was diluted with warm normal saline to make a 1 per cent solution.

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This anæsthetic was the most difficult to manage because of the length of narcosis produced by an anæsthetic dose and the difficulty of judging depth. It was found that a dose of 0.6 ml. of the 1 per cent solution often produced sleep lasting well over half an hour, while less than this dose frequently did not anæsthetise the rat at all. An inducing dose of 0.6 ml. was therefore given to all except the lightest rats (under 120 gm.), which were given 0.5 ml. A much grosser degree of reflex movement on stimulation than after nembutal was taken as the indication for injecting a supplementary dose. After anæsthesia these animals remained sleepy for several hours, although responding actively to stimulation.

It was noted that at post-mortem on rats dying during pentothal anæsthesia, there was frequently a considerable amount of fluid in the peritoneal cavity.

Acknowledgments

I am indebted to Messrs. Bengers, Ltd., Holmes Chapel, for allowing me the facilities of their laboratory and for providing the experimental animals used. My thanks are also due to Dr. F. Fletcher, of Bengers Laboratories, for much useful advice, and to Prof. J. G. Wright for helpful criticism.

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FURTHER OBSERVATIONS ON EPIZOOTIC CEREBROSPINAL NEMATODIASIS:

1. Chemotherapeutic Control of the Disease by 1-Diethylcarbamyl-4-Methylpiperazine Citrate: Preliminary Field Trial

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Introduction

THE introduction of the cotton rat (*Sigmodon hispidus*), naturally infected with filaria (*Litosomoides carinii*), in 1945, for the evaluation of drug activity supplanted all other *in vivo* methods in the search for new antifilarial chemical agents. A comprehensive account of all work done in the U.S.A., by many workers, leading to the discovery of a 1-diethylcarbamyl-4-methylpiperazine hydrochloride, as an unrivalled active filaricide, was presented at a conference on " The Chemotherapy of Filariasis " in 1947 (published by New York Academy of Sciences, 1948). Since then further evidence has accrued and the early promise of efficacy against *Wuchereria bancrofti* has fulfilled expectations. In Japan, the citrate salt of the same compound has been tried by some workers

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against ascariasis—Hano, *et al.* (1950), and Mitsufuji, *et al.* (1950)—and against human filariasis (*Wuchereria malayi*), in which remarkable results were obtained by Hayashi, *et al.* (1951), on Hachijo Kojima Island. There was thus a lead to test this same drug against epizootic cerebrospinal nematodiasis in sheep, goats and horses, caused by *Setaria digitata,* concerning which many previous papers have now appeared (Yamagiwa and Shoho [1944], Tanaka, *et al.* [1945]), Shoho [1952], Innes [1951], Innes and Shoho [1952], and Innes, Shoho and Pillai [1952]). From all the latter no further doubt should remain that (*a*) this neuroparalysis is a common disease (perhaps in many parts of the world remote from the Orient, as Innes, Shoho and Pillai indicate), (*b*) the pathology is unique and distinctive (a focal encephalomyelo-malacia), and (*c*) that nematodes play the primary causative role.

The following is a summary of the results of a pilot type of field trial, undertaken to ascertain the value of the drug in a prophylactic and curative direction—the latter at the *very early onset* of nervous signs. Publication at this stage, tentative though it may appear to be, may stimulate work along similar lines in other parts of the world concerning this helminthic neuroparalysis and its control, for it may be proved in time (as Innes and Shoho believe) to have an importance far beyond that at present imagined.

Field Experiments and Methods

The trial was made in the Daté district of Fukushima prefecture, with the active interest and financial aid of the local Corporation of Animal Husbandry. All sheep and goats in this district are kept in shelters with small compounds, for the animals are not grazed freely, as in other countries. In the summer season they are fed on grass, and sometimes on the remnants of mulberry leaves—primarily used for silk-worm feeding. All foals and yearlings (Arabian or thoroughbreds) are kept separately in stables, with outside grazing. In nearly all farms, cattle (native breed crossed with Brown Swiss) are kept adjacent to sheep, goats and horses. The cattle, as described elsewhere, are carriers of the adult *Setaria digitata,* and in the summer and autumn the cattle are the hosts for transmission of the microfilaria through mosquitoes to sheep, goats and horses (unnatural hosts), which in turn may become victims of the neural nematodiasis.

Clinical experience against human filariasis indicated that repeated small daily doses of the drug seemed the ideal method, but with large numbers of animals in the field this was impossible. In any case, the first concern was to determine whether the drug had any activity against the *Setaria* wandering from subcutaneous tissue to, and in, the craniovertebral cavity, for microfilaria are not present in the blood of the unnatural hosts—sheep, goats and horses. Yet, consideration must be given to the possible coincidental occurrence of microfilaria of *Setaria equina.* A preliminary note by Sasa, *et al.* (1951), indicated that a daily dose of 100 mg./kg. body-weight for five successive days did not produce toxic signs in goats. Thus, a single dose of 20 mg./kg. was chosen arbitrarily for prophylactic trials, but in some animals 40 mg./kg. was given. For therapeutic work 40 mg./kg., on one or two successive days, was given

to sheep and goats, and up to 80 mg./kg., on two successive days to valuable foals (in the latter after blood examination revealed an absence of microfilaria of *S. equina*). The drug was dissolved in an adequate quantity of water, and given by stomach tube. It was first thought that in ruminants, and in horses (with such an anatomically large cæcum), slow absorption of the drug might be obtained by one large dose. Later it was found in carrier calves that rapid absorption occurs from the ruminant stomach, for a filaricidal action on microfilaria of *S. digitata* in the blood was noted within three hours after dosing.

The reports of the Japanese Commission on Research of Lumbar Paralysis in Korea (1939-44), in which the original discovery of nematodal cause for the disease was reported, showed that in sheep (imported into Korea from Australia) signs of neuroparalysis appeared about one month after being landed in Korea. A comparable latent period was noted in their transmission experiments, while Itagaki, *et al.* (1946-47) noted a range of 16 to 66 days in the case of experimental horses; hence it was assumed that the shortest incubation period might be about 16 days. For our purposes in assessing activity of the drug as a prophylactic it was decided to use a period of 20 days after dosing, after which a second or third medication might be given.

Results of the Trials

(A) Prophylactic.

Four separate groups of prophylactic trials were made, and the results are tabulated below. Comments on each trial are made and the results thereafter discussed.

TABLE I.

Trial.	Village.	Numbers of Sheep Treated. Nos.	No. of Cases.	Untreated Controls. Nos.	Cases.
1	Danzaki	161	0	119	0
	Handa	58	1	62	0
	Total	219	1	181	0
2	Ookido (Aug. 31) ...	78	1	85	?
	Handa (Sep. 1) ...	30	0	90	3
	Danzaki (Sep. 2) ...	87	1	193	11
	Ishido (Sep. 3) ...	36	1	236	?
	Total	231	3	604	14+
3	Ishimoda (Sep. 10) ...	19	0	50	3
	Morieno (Sep. 12) ...	68	0	182	?
	Total	87	0	232	3+
4	Handa (Sep. 21) ...	29	0	91	3
	Danzaki (Sep. 22) ...	67	1	231	1
	Total	96	1	322	4
	Grand Total ...	633	5	1,339	21+

The first trial was made between August 10 and 13, but the relatively cool early and middle summer did not predispose to a large number of paralytic cases occurring. In the second trial, the sheep used included those in the Danzaki and Handa locality treated in the first trial. Forty-eight of the 78 sheep in Ookido area received 40 mg./kg.; some of the control animals in addition had been previously treated by intravenous antimonial compound by others, which rather vitiated the result of no cases (?) being found. Further, the one case noted in the treated group (Ookido) showed signs of slight inco-ordination and paralysis on the day treated; two daily successive treatments of 20 mg./kg., and 40 mg./kg., resulted in dramatic recovery, and it was able to stand on the fourth day; no further signs were thereafter noted. The case in the treated group (Danzaki) showed signs on the seventeenth day ,and again similar improvement occurred by further dosage of 40 mg./kg. In the sheep in Danzaki and Handa taken together there was thus a total of 14 cases of paralysis in the control sheep within 20 days, as opposed to only one in the treated, which might speak in favour of the value of repeated drug administration within two to three weeks. In the third trial the results were somewhat confusing, as some of the untreated sheep had also been given previous antimonial preparation by the farmers. In the fourth trial the only case in the treated group occurred on the seventh day after dosing, and this animal recovered completely after being given 40 mg./kg.

Comment.—All field trials of this nature, without adequate data on pharmacology of the drug, and without the constant supervision of the animals as can be effected in Experimental Field Stations, can never give wholly satisfactory results. Yet it is contended there is sufficient evidence to indicate that the drug has some prophylactic activity, certainly to justify a further, wider, and more carefully-controlled experiment. The prompt disappearance of nervous signs in the four cases treated therapeutically with 40 mg./kg. might indicate that this dose would be more effective when given also prophylactically. Perhaps the first need, therefore, is for adequate pharmacological studies of the drug on ruminants and horses. If the results of the second and third trials in Handa, Danzaki and Ishimoda are separated from the whole and considered, perhaps they take on more significance in view of what was known (by personal investigation) regarding the incidence of the disease. In this area from about 1942 it has been estimated that about 6-7 per cent of sheep were affected annually, occurring from about the middle of August to the middle of October. Hence, in this second and third trial, out of a total of 136 treated sheep only one case was found, as against 17 cases in 333 untreated control sheep, and some of the latter had also been given antimonials; the percentage in the latter (5.1 per cent) thus corresponds to what was known about annual natural incidence (6-7 per cent).

Experiments with Foals

Cerebrospinal nematodiasis due to immature *Setaria digitata* in horses and foals is relatively common in Japan. In adult animals, however, neurological signs may not appear, or may not be discernible, as was shown first by the

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important findings of Tanaka, *et al.* (1945); in a routine neuropathological survey of the brains of 100 old serum horses, they found minute lesions with parasites in the brains of seven animals which had shown no neurological signs in life. As Innes, Shoho and Pillai point out, this may not be considered astonishing, for in adult animals with a voluminous nervous system such as in the equine tribe—as the lesion of nematodiasis is about the same magnitude as in other affected species, sheep and goats—much would depend on the location of the nematode (and ensuing lesion) in the brain or cord. Affected foals and yearlings conversely show unmistakable neurological signs—noticeable to laymen—which have an exact clinical counterpart in the descriptions of both wobblers of foals and yearlings in the U.S.A., and so-called Kumri of horses in India and Burma. Innes, *et al.*, have also pointed out that, in effect, these may be identical diseases, and that the idea of nematodal cause in the case of Kumri can be traced back to antiquity, though accurate pathological studies have still to be made and reported.

The equine form of cerebrospinal nematodiasis usually occurs at a slightly later part of the season than in the case of the disease in sheep and goats—i.e., usually around the middle of September. Further, ocular filariasis of horses in Japan (mostly due to *Setaria digitata,* according to the recent work of Kume, *et al.* [1951]) appears even later still, about the last weeks of October, when the incidence of neural nematodiasis has begun to subside. All this might be understandable, for in horses it might take the infective larvæ much longer to undertake the " 10-day Cook's tour " (referred to by Chandler to describe larval tissue migration) from subcutaneous inoculation to nervous system or eye. (In Itagaki's work a period of 66 days occasionally elapsed.) Obviously, the actual seasonal date of infection by the mosquito vectors must be the same for all domestic animals. Thus, for prophylactic purposes it was decided to administer the drug on September 20.

Seven valuable foals were given the drug at a dosage varying from 40-60 mg./kg. on September 20. No toxic effects were observed. No signs of either neural or ocular nematodiasis were observed in any animal by the end of November. The small number of animals used makes any conclusions almost worthless, except for two facts, (*a*) the results of the trials in sheep, and (*b*) more important still, the dramatic curative effect of the drug in two unmistakable cases of neural nematodiasis in foals described below.

(B) THERAPEUTIC.

Before discussing the results of therapeutic treatment it is necessary to refer readers to a previous paper by Innes, Shoho and Pillai (1952), in which the neuropathological nature of the disease is fully described and illustrated. The damage in the nervous system produced by the wandering, developing, immature nematode is essentially traumatic disintegration, sometimes hæmorrhagic, leading to liquefaction, and perhaps cavitation. It is also known that there is a variable involvement of the meninges and nerve roots, shown by the presence of inflammatory cellular exudate in which eosinophils may predominate. We know that there is a latent period of some weeks between the time the mosquito bites

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the animal, transmits infective larvæ, and appearance of nervous signs. What we do not know is the minimal amount of damage or irritation (meningeal, radicular or nervous) required before animals show neurological signs, other than those of complete flaccid paralysis. This complex question of correlation of clinical neurological signs and nervous lesions is indeed a constantly recurring complex one in the whole field of comparative neuropathology. It might, however, be imagined that the early presence of the immature worm in the cranio-vertebral cavity might produce, initially, inflammation of the meninges and nerve roots, and consequent irritation, with an insidious onset of inco-ordination and ataxia, and which might not be succeeded by actual nematodal invasion of nervous tissue, with its resultant malacic process, for a few days. This might, therefore, be one reason why it should be possible to cure affected animals, provided the cases are in a *very early* stage. We know from other directions in experimental neurophysiology that in animals such as sheep even a massive lesion may be compensated in time, particularly if such a lesion is not located in vital parts. Lesions in the lower part of the lumbar spinal cord would certainly not cause such permanent severe neurological impairment as at higher levels. Obviously, once the lesion has reached the malacic liquefactive stage no complete cure by the drug would be possible, i.e., in an animal with a history of paralysis of many days' or weeks' duration.

(1) *Sheep and goats.*—A total of 64 sheep and four goats have been treated, and in no case was any toxic action, due to the drug, observed. In general, the efficacy of the drug was such that nervous symptoms disappeared within one or two days. Out of 11 ovine cases treated by a single dose of 40 mg./kg., 10 showed prompt recovery and with no residual signs; it is manifest that a single dose at this level does kill the wandering nematode. In the remainder, more than one dose was employed with no better results. In a few, repeated doses did not prevent residual neurological symptoms, but in these cases the course of the disease before treatment had been longer. Experience in goats was less, but in three animals affected and obtained in an early stage comparable dramatic effects were obtained by 40 mg./kg. In a fourth caprine case, 40 mg./kg. was given for six successive days with increased appetite and obvious locomotor improvement within two days, but this animal again showed nervous signs on the tenth day. The question arose as to the possibility of the animal having been reinfected, for it is known that this phenomenon does occur. A second course of treatment on the tenth day resulted in complete recovery.

(2) *Foals.*—The therapeutic results were equally as striking as in the case of the sheep. In the first case, nervous symptoms were noted on September 14 (many years ago this foal would certainly have been diagnosed as virus encephalitis). The animal was seen personally on September 16 and a diagnosis of cerebrospinal nematodiasis made. On September 16, 20 mg./kg. was given orally, 10 mg./kg. on the 17th and 18th, and 30 mg./kg. on the 19th and 20th. The foal was still ataxic on the 18th, but it was able to stand, while by the 26th it had recovered, except for a slight residual weakness of the right hind leg. The second case, found to be markedly ataxic on September 22, was given 50 mg./kg.

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on the 23rd, and 80 mg./kg. on the 24th; some improvement was seen on the 25th, and by the 28th the animal was normal, without any neurological signs whatever.

General Discussion

Viewed in the cold light of science, or in that of those who will only believe statistical evidence, the results given above may not appear of profound significance. Yet it must be reiterated that the object of such a pilot trial was to obtain merely preliminary indication of some activity of this new filaricide, already proved to be efficacious against human filariasis. In the opinion of my collaborator in the U.S.A. (Dr. Innes), the results are just as promising, and rest on the same insecure statistical basis, as in the case of the very early work on phenothiazine as an anthelmintic, or on copper as an antiswayback measure. The very nature of the disease and its filarial cause should be remembered, for then dramatic therapeutic and prophylactic results, provided sufficient dosage was given, cannot be viewed as extraordinary.

In Japan, for several years other chemotherapeutic agents have been tried, especially arsenical and antimonial preparations, but all were found to be too toxic. The advent of the compound used in these trials, which was discovered in Lederle Laboratories in the U.S.A., opens up an entirely new vista in the problem of the control of this disease in Japan, Korea and Ceylon—and elsewhere if the convictions of Innes, Shoho and Pillai are confirmed. Theoretically, as in other fields of parasitic disease, perhaps the ideal method of control would reside in treatment of the natural hosts (cattle), harbouring the adult *Setaria* and acting as carriers and reservoirs of infection. Personal observations have already indicated that a marked drop in the microfilarial count in carrier cattle can be achieved within a matter of hours by a single dose of 100 mg./kg., and no doubt might be completely eliminated by adequate dosage; considerably more work is necessary, first in a pathological direction and then with a sufficient number of "microfilaria carrier" cattle. At this stage there is no need to consider the most suitable mode of administration, either as a prophylactic for sheep, goats or horses, or a drug to eliminate the cattle reservoirs of infection; for practical field purposes individual dosing would be a great drawback.

Conclusions

Data are presented which indicate that 1-diethyl-carbamyl-4-methylpiperazine citrate possesses a filaricidal activity in the disease of domestic animals (sheep, goats and horses) now known as epizootic cerebrospinal nematodiasis. In the dosage employed in the field trials no toxic reactions were noted. The results of prophylactic trials are certainly of sufficient significance to support ideas for comparable projects on a far more ambitious scale in Japan, Ceylon, and elsewhere. The therapeutic results in the light of personal experience of a large number of cases are considered to be dramatic.

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RECENT TREATMENT OF A CASE OF BURSATTEE

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Subject

ON 25.1.1951, a she-donkey about 5 years old was brought to the Faculty's hospital for treatment.

History

On questioning the owner, it was revealed that the animal had been brought to the same hospital the previous year suffering from large ulcerated wounds in the shoulder and neck. Those lesions healed up after two months of ordinary treatment. This year, in the exact time, the animal showed symptoms of similar lesions on the hock, eyelid, and fetlock. It began as swellings, which later on became harder. In about two weeks' time the skin covering the diseased areas sloughed, exposing an extensive ulcerated surface. The owner stated that the lesions were a fortnight old and he had not tried any treatment except ordinary antiseptics.

Clinical Examination

On examination, sores were found on the right upper eyelid, the inner aspect of the hock, and the posterior aspect of the fetlock of the near hind. The hock lesion was extensive, involving the subcutaneous tissue, overlying skin and in which were embedded small fibrous tumours containing no pus. The lesion was hot and painful to the touch, with ulcerated surface, thickened in its margins, and covered by yellowish-red granulations weeping an amount of thin, yellowish liquid and having a tendency to spread, attaining a large area. The eyelid and fetlock lesions were small. Respiration, temperature, and pulse were normal and no constitutional symptoms could be detected.

Diagnosis

As these superficial ulcerative and raw wounds showed seasonal occurrence, and as the parts of the body involved were the most frequent sites of bursattee, and also from the above history and symptoms, the condition was diagnosed clinically as "bursattee." Microscopical examination was negative to fungus and larvæ.

Ætiology

Authors differ as to the cause of bursattee: (1) some claim that it is due to worm embryos (*Habronima musca*); and (2) others state that it is caused by a fungus.

Ordinary Methods of Treatment

Treatment in common use was based on the following:—

(1) Removal of the fibrous tumours surgically;

(2) Actual cautery or the application of caustics;

(3) Administration of arsenical preparation;

(4) Application of biniodide of mercury;

(5) Application of a mixture of equal parts of lactic and salicylic acids together with two parts of collodion; and

(6) Painting with 10 per cent alcoholic solution of picric acid or zinc chloride.

Treatment Adopted in This Case

On 27.1.1951:—

At 11 a.m.—*Intravenous injection:* (1) 1,000,000 U.O. of Acticilline forte "R.I.S.T." (two vials, each containing penicillin G. procaine crystalline 300,000 U.O., pencillin G. potassium crystalline 200,000 U.O.); (2) 10 gm. sulphamezathine (ampoules). *Electuary:* 20 gm. sulphamezathine powder and 15 gm. sodium citrate.

At 5 p.m.—*Electuary:* 30 gm. sulphamezathine powder and 15 gm. sodium citrate.

On 28.1.1951:—

At 11 a.m.—*Intravenous injection:* As on 27.1.1951. *Electuary:* 20 gm. sulphamezathine powder and 15 gm. sodium citrate.

At 5 p.m.—*Electuary:* 25 gm. sulphamezathine powder and 15 gm. sodium citrate.

On 29.1.1951:—

At 11 a.m.—*Intravenous injection:* As on 27.1.1951. *Electuary:* 15 gm. sulphamezathine powder and 15 gm. sodium citrate.

At 5 a.m.—*Electuary:* 20 gm. sulphamezathine powder and 15 gm. sodium citrate.

On 30.1.1951:—

At 11 a.m.—*Intravenous injection:* (1) 1,000,000 U.O. of Acticilline forte. (2) 5 gm. sulphamezathine (ampoules). *Electuary:* 15 gm. sulphamezathine powder and 15 gm. sodium citrate.

At 5 p.m.—*Electuary:* 15 gm. sulphamezathine powder and 15 gm. sodium citrate.

On 31.1.1951:—

At 11 a.m.—*Intravenous injection:* (1) 1,000,000 U.O. of Acticilline forte. *Electuary:* 15 gm. sulphamezathine powder and 15 gm. sodium citrate.

At 5 p.m.—*Electuary:* 10 gm. sulphamezathine powder and 15 gm. sodium citrate.

On 1.2.1951:—

At 11 a.m.—*Electuary:* 10 gm. sulphamezathine powder and 15 gm. sodium citrate.

At 5 p.m.—*Electuary:* 5 gm. sulphamezathine powder and 15 gm. sodium citrate.

On 2.2.1951:—

At 11 a.m.—*Electuary:* 5 gm. sulphamezathine powder and 15 gm. sodium citrate.

At 5 p.m.—*Electuary:* 3 gm. sulphamezathine powder and 15 gm. sodium citrate.

Result

Improvement was noticed after five days (31.1.1951) from the commencement of the treatment, when healing started from the periphery of the hock lesion, where crusts were formed and sloughed, leaving a healthy-looking area. Eyelid and fetlock lesions were completely cured. On 3.2.1951, administration of all medicines was stopped. However, non-medicated vaseline was smeared once daily to soften the crusts already formed on the central part of the lesion.

Discussion

It is proved beyond doubt that the case dealt with is one of bursattee, for the following reasons:—

(1) The position of the lesions.

(2) The seasonal recurrence of the disease, in spite of the fact that similar lesions in the animal were treated in the past by ordinary methods with apparent success.

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The treatment adopted in this case has saved the trouble of applying the drastic measures in common use, such as excision of the lesions, or application of actual cautery or caustics.

Summary

(1) A case of bursattee in a she-donkey is described.

(2) A review of the ordinary treatment is mentioned.

(3) Treatment of bursattee with penicillin and sulphamezathine, without any specific topical application of the drugs advised before, proved very successful.

(4) Recovery was achieved in a very short time (seven days), compared with the long time taken when using the ordinary methods of treatment reviewed.

(5) This treatment, based on the use of up-to-date drugs, has done away with the drastic measures adopted in treating bursattee.

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NOTICE

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